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Fungal Quality of Fresh Shrimp sold at Ndibe Beach in Afikpo, Ebonyi State

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Abstract

This study was conducted in the Department of Applied Microbiology with the aim to obtain the fungi qualities of shrimps from Ndibe beach, Afikpo North LGA, Ebonyi State Nigeria using pour plate method (after serial dilution) and lactophenol staining . The isolated organisms were identified and characterized following the standard microbiological methods. The result of total fungi count revealed that the average fungal colonies count from different sample parts are 2.07 x 10^6, 0.39 x 10^6 and 0.79 x 10^6 respectively for head, body and tail. The result of the isolates and identification of fungi from different parts of the shrimp samples from three different vendors revealed that five fungi isolates were identified. These isolates include: *Aspergillus* species, *Penicillium* species, *Mucor* species, *Fusarium* speciesand *Rhizopus* species. The result percentage frequency of occurrence revealed that *Aspergillus* spp 8(25%) 7(21%) 7(27%), *Penicillium* spp 7(21.9%) 5(20%) 6(23%), *Mucor* spp 5(15.6%) 5(20%) 6(23.1%), *Fusarium* spp 8(25) 4(16%) 4(154%) and *Rhizopus* spp 4(12.5%) 4(16%) 3(11.5%) respectively for vendors A, B and C. This shows that *Aspergillus* species have the highest frequency occurrence with *Rhizopus* species having the lowest frequency occurrence. In conclusion, the presence of these Fungi in the Shrimps is attributed to contamination from the environment and the shrimp handlers (mongers) or as normal flora of the shrimp which unfortunately happens to be opportunistic pathogens or pathogens of humans. The maintenance of high personal and environmental hygiene as well as proper heating and cooking will improve fresh shrimp quality and prevent food-borne diseases.

INTRODUCTION

Brown shrimp is a small free-swimming edible crustacean with 10 legs. It is basically marine commonly found in estuaries and along coastal waters. They are widespread, and can be found near the seafloor of most coasts and estuaries, as well as in rivers and lakes. There are numerous species, and usually there is a species adapted to any particular habitat [1]. Most shrimp species are marine, although about a quarter of the described species are found in fresh water [2]. Marine species are found at depths of up to 5,000 metres (16,000 ft) [3], and from the tropics to the Polar Regions.

In Nigeria, there is a large number of public frozen seafood processing plants distributed along the country, where a considerable number of people buy their frozen seafood products daily. Serious consequences relating to national productivity and development can arise from lack of hygiene and sanitation in such outlets Studies investigating the agents that affect this invertebrate are rare and only a few viruses, bacteria and protozoa are known to cause disease in these crustacea [4].

These edible crustaceans are composed of 41% (w/w) high value protein and omega-3 fatty acids which have anti-inflammatory effects and are able to prevent the formation of blood clots)[5]. They are also important sources of vitamin D and B12 needed to keep low levels of

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homocysteine - a molecule that can directly damage blood vessel walls and is considered a significant risk factor for cardiovascular diseases[5]. Fish and shellfish are one of the most important sources of animal protein and have been widely accepted as a good protein source and other elements for the maintenance of healthy body [6]. It also provides a good source of high quality protein and contains many vitamins and minerals.

Shrimps, apart from being of great nutritional value to the consumer; also serves as an ideal culture medium for microbial growth, which connotes spoilage [5]. Despite all the benefits associated with shrimps, one thing still remains clear and yet unsolved; shrimps are highly prone or susceptible to rapid deterioration immediately after harvest.

Microorganisms are undoubtedly responsible for spoilage of shrimps and these microorganisms include bacteria and fungi, both the heterotrophic and pathogenic forms. Molds which belongs to fungi, grows rapidly on food (shrimp) when held under moist conditions. These organisms take advantage of the moist condition available and through the aid of enzymes which they possess, they weaken and penetrate the protective outer layer of the shrimp and cause spoilage [5].

It is almost always possible to detect a range of human pathogens on any shrimp that has not received any microbiocidal treatment [7]. Some of these pathogens may constitute part of the normalflora on the shrimp or be present as a result of unavoidable contamination [8]. Also, a normal microflora of humans can cause spoilage of shrimp by way of contamination through handlers, as they touch the shrimp with bare hands in the bid of harvesting and selling the shrimps. Also shrimp spoilage based on microbial origin, originates mainly from the shrimp's environment (which is aquatic) and in this environment, contamination may occur as a result of the presence or introduction of microorganisms through faecal means into the water bodies.

Fresh brown shrimps sold in the common markets in Afikpo do not undergo any form of microbiocidal treatment, and they are not iced before sale. The shrimp mongers also handle them with bare hands during transactions. Some shrimp consumers cook the shrimps whole (that is, including the head, exoskeleton and telson/uropods); others remove the head, exoskeleton and telson/uropods of the shrimps before cooking (only flesh) while some consumers after removing the head, exoskeleton and telson/uropods, grind them and add the paste to the soup or sauce.

There is the need to ascertain the microorganisms (fungi) associated with these various parts of shrimps and the health hazards associated with the types of microorganisms. The knowledge of the types of microorganisms involved in their spoilage will also help to control shrimp spoilage[5].

Obire and Minimah, (2005) reported that fungi isolated from fresh brown shrimps (*Penaeusaztecus*) purchased from three different markets in Port Harcourt using standard mycological methods gave total counts of heterotrophic fungi range from 2.0 x 104 spore forming units per gram (sfu/g) to 7.1 x 104sfu/g, while the total counts for pathogenic fungi range from 1.7 x 104sfu/g to 7.1 x 104sfu/g [9]. The heterotrophic fungi in the H/E/T (head, exoskeleton and telson/uropod) before deterioration and about deterioration were: *Aspergillus*

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clavatus, Aspergillus flavus, Penicillium spp, *Rhizopus* spp, *Rhodotorula* spp, *Aspergillus flavus, Mucor hiemalis, Penicilllium sps, Rhizopus* spp , *Rhizopus stolonifer*, Yeast spp [9].

The above suggests that most shrimps sold in the market may be sources of microbial food poison and intoxication hence, they are counterproductive by being responsible for public health hazard and loss of revenue. The presence of these fungi in the shrimps is attributed to contamination from the environment and from shrimp handlers (mongers). Also, some of these fungi are normal flora of the shrimp which unfortunately happens to be opportunistic pathogens or pathogens of humans.

MICROBIAL IMPORTANCE OF SHRIMPS

They contain high quality protein and other essential nutrients which can be low in saturated fat and may contain omega-3 fatty acids. In fact, a well-balanced diet that includes a variety of fish and shellfish can contribute to heart health and children's growth and development. But, as with any type of food, it's important to handle seafood safely in order to reduce the risk of foodborne illness [10,11,12,13,14]

Seafood is an extremely perishable commodity and quality loss can occur very rapidly after catch [15,16]. Shrimp is one of the most delicious seafood and is part of almost every nation's traditional meal. The source of pathogenic bacteria may be from natural and unhygienic handling of shrimp by the workers [17]. Seafood can be exposed to a range of hazards from the water to the table. The hazards can involve bacteria, viruses, parasites, natural toxins and chemical contaminants. When these processed frozen seafood products are consumed raw, there is the likelihood of endangering the health of the consumers especially, when the micro- organism present include pathogenic ones [18]. Unsafe water used in processing seafood products pose a global public health threat, placing person at risk for a host of diarrhoea and other diseases [19]. Insufficiently iced and improper storage of shrimp at higher temperature enhances the growth of microorganisms responsible for microbiological changes [20]. The microbiological safety of food is achieved by as far as possible ensuring the absence of pathogenic microorganisms and by all means preventing their multiplication [21].

Although diseases in feral populations of shrimps have not been seriously studied, there is an awareness of mycoses in cultured shrimps, owing to losses incurred than interests in mycotic afflictions. Karunasagar *et al.* (2004) suggested that nearly 500 fungal species have been isolated from marine and estuarine environments, of which a few are pathogenic to shrimps [22].

Mostly, larva stages of shrimps are affected by *Lagenidium callinectes* and *Serolpidium* spp. Clinical signs such as lethargy and mortality due to fungal afflictions can be detected in protozoa and Mysis stages. Usually, fungal spores and mycelia can be observed in affected tissue especially gills and appendages. Mycoses are problematic for larval stages in many hatcheries in India. (Gopalan *et al,* 1998) reported *Lagenidium marina* and *Serolpidium parasitica* infections in *P. monodon*[23]. Ramasamy *et al.* (2006) reported mortality in P. monodon larval at nauplii, zoea and mysis stage [24]. Fusariosis and black gill disease caused by *Fusarium* spp may affect all developmental stages of Penaeid shrimp. *Fusarium* spp (*Fusariumsolani* and *Fusarium moniliformae*) are opportunistic pathogens

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that may lead to high mortalities. Disease is noticed in ponds where water management quality is poor. Fungal hyphae can be detected in affected animal tissue using light microscopy [24].

MATERIALS AND METHODS

STUDY AREA

The study was carried out in Ndibe beach, Afikpo North L.G.A in Ebonyi State South East Nigeria. Geographically it is located on latitude 6^0N and longitude 8^0E of the Greenwich meridian with the estimated population of 156,611 according to the Nigeria 2006 census.

COLLECTION OF SAMPLES

60 grams of fresh shrimp each were purchased from four different vendors within Ndibe beach market. The Shrimps were immediately transported in sterile plastic bags with ice block to the Microbiology laboratory unit of Ebonyi State University for identification and analysis.

SAMPLE IDENTIFICATION

The fresh shrimp sample was identified as *Macrobranchium vollenhouenii* by Mr. Uho Cosmos, a taxonomist in Applied Biology Department of Ebonyi State University.

ANALYSIS PROCEDURE

The shrimp from each of the three vendors were aseptically divided into head, body and tail with a surgical blade. Each part was pounded using a sterile porcelain mortar and pestle and kept differently. A 10g portion of each (head, body and tail) from the different vendors was weighed out and homogenized with 90ml of peptone water for 10mins. 1ml each of dilution five (5) of the homogenate was pour plated on Sabouraud Dextrose Agar (SDA) and incubated for 72hrs at 37°C. Discrete colonies were counted and subcultured onto a fresh SDA plates inhibited with chloramphenicol and incubated for 3-4 days after which the plates were examined.

CHARACTERIZATION AND IDENTIFICATION OF FUNGI ISOLATE

The fungi isolates were identifed on the basis of colonial morphology followed by microscopic examination after lactophenol staining.

LACTOPHENOL STAINING

Little portion of the fungal isolate was mixed in a drop of lactophenol cotton blue stain made on a clean grease free microscopic slide using sterile wireloop, a smear was made and covered with a sterile cover slip. It was observed under the microscope using x10 and x40 objective lenses for detection of features as hyphae, mycelium and spore [25].

RESULTS

The result of total plate count of fungi from fresh shrimp collected from Ndibe beach in Afikpo North Local Government Area of Ebonyi State Nigeria is shown in Table 1. It revealed that the average fungal colonies count from different sample parts are $2.07x10^6$, $0.39x10^6$ and $0.79\ x10^6$ for head, body and tail respectively.

The result on the descriptional features of the fungal isolates from the shrimp is shown in Table 2. It showed that five (5) fungal isolates were identified which include *Aspergillus* species , *Fusarium* species, *Mucor* species, *Penicillum* species and *Rhizopus* species. All the fungal isolates are Lactophenol positive.

The result on the percentage frequency of occurrence of the fungal isolates from the shrimp collected from three vendors is shown on table 3. It revealed that for vendor A, *Aspergillus* speciesand *Fusarium* species

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have the highest percentage frequency of 8(25%) respectively while *Rhizopus* species has the lowest percentage frequency of 4(12.5%).

For vendor B, *Aspergillus* species have the highest frequency of 7(28%) while *Fusarium* speciesand *Rhizopus* species have the lowest percentage frequency of 4 (16%) respectively.

For vendor C *Aspergillus* species *have* highest percentage frequency of 7(27%) while *Rhizopus* species have the lowest percentage frequency of 3(11.5%). In summary, *Aspergillus* species have the highest frequency and *Rhizopus* species have the lowest frequency in all the samples surveyed.

Table 1: Total plate count of fungi from fresh shrimp

Source	No of colonies counted	Fungi count x10^6 (cfu/ml)
HA	250	2.50
HB	186	1.86
HC	186	1.86
BA	36	0.36
BB	32	0.32
BC	50	0.50
TA	120	1.20
TB	80	0.80
TC	36	0.36

Average fungi count for head, body and tail are 2.07 x 10^6, 0.39 x 10^6 and 0.79 x 10^6 respectively.

Key:
HA - Head from vendor A
HB - Head from vendor B
HC - Head from vendor C
BA - Body from vendor A
BB - Body from vendor B
BC - Body from vendor C
TA - Tail from vendor A
TB - Tail from vendor B
TC - Tail from vendor C

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Table 2: Descriptional features of fungi isolates

S/N	Colour	Consistency	Microscopy features	Lactophenol cotton blue	Suspected fungal isolate
1	Nearly green	Grey fluffy mycelium	chains of single cell Conidia spherical, rod and regular in size	+	*Penicillium* species
2	Greenish brown, white to black	Rough walled forming long dry chain	Flask Shaped Phialides, Conidia in chain and branching at 45^{0}c	+	*Aspergillus* species
3	Whitish to grayish	Dark, rough raised	Brand hyphal freely spread irregular and ribbon like	+	*Mucor* species
4	Pale brown to dark brown	Fluty, rose or purple pigment on surface	Irregular Conidiosphore and in Clusters	+	*Fusarium* species
5	Black	Cotton like	Irregular sporangiosphore, contains root like Rhizoids	+	*Rhizopus* species

Key: + = positive

Table 3: Percentage frequency of occurrence of fungal isolates from fresh shrimp from vendor A, B and C.

Vendors	*Aspergillus* species	*Penicillium* species	*Mucor* species	*Fusarium* species	*Rhizopus* species
A	8(25%)	7(21.9%)	5(15.6%)	8(25%)	4(12.5%)
B	7(28%)	5(20%)	5(20%)	4(16%)	4(16%)
C	7(27%)	6(23%)	6(23.1%)	4(154%)	3(11.5%)

DISCUSSION

In the present study a number of fungal species were found to be associated with the head, body and tail of shrimps. The different fungal isolates include: *Aspergillus* spp, *Mucor* spp, *Penicillium* spp, *Fusarium* sppand *Rhizopus* spp. The fungi count recorded in the shrimps are ranged from 0.36 x 10^{-6}cfu/ml to 2.5 x 10^{-6}cfu/ml. This is similar to the report of Obire andMinimah (2005), whichisolated similar fungi species from fresh brown shrimps (*Penaeus aztecus*) purchased from three different markets in Port Harcourt [9]. The high fungal count is an indication of gross contamination of the environment where the shrimps were harvested and of the ecological niche where the fungi has developed. The results of fungal counts were almost exactly the same with that reported by Obire andMinimah (2005) [9]. This is not surprising because the distance between the vendors in Ndibe beach market is less than 20 m.

Among the shrimp samples, *Aspergillus* spp has the highest frequency in all parts of the shrimps. It is also the most powerful fungi to produce Aflatoxin which are mycotoxin with carcinogenic potential.

The potential for disease outbreaks attributed to sea food ingestion has been recognized for over a century [26]. It is important to state here that most of the fungi are potential pathogens. Some diseases caused by these fungi in humans are

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Aspergillosis, Liver tumour disease, Fungal balls in the lungs, Chronic productive cough and haemoptysis, bronchial asthma, infection of the ear or Para nasal sinuses, fungal cells within histiocytes, necrosis and eventual abscess formation, multiple brain abscesses and Varicose dermatitis, a chronic human [8, 26, 27]. Others include rhino cerebral mucormycosis, infection of the nasal turbates and Para nasal sinuses spreading rapidly to the eyes and brain; necrosis and thrombosis and invasive mucormycosis [28].

CONCLUSION AND RECOMMENDATION

The presence of fungal species in the shrimps is also attributed to contamination from shrimp handlers or processors or sellers (mongers). The fungi isolated from the shrimps in this study are potential pathogens and they are capable of causing chronic illnesses in humans upon ingestion of food contaminated by them. Owing to the health hazards associated with the fungi isolated from the shrimps used for this study, it is important that shrimps and other sea food product be properly and adequately cooked. Also unfavorable conditions should be created to prevent fungal growth, examples of such conditions are: regulation of the water holding capacity of the food (aw), temperature and pH Adjustments in these areas will help prevent mycotoxin contamination of the shrimp as moulds have strains which produce toxins that are capable of causing serious chronic illnesses (e.g. liver tumor caused by *A. flavus*) in humans, if consumed.

Being that some fungi contaminated the shrimps through shrimps handlers or processors, there is there- fore the need for the adoption, practice, and maintenance of good personal hygiene as regards handling of the shrimp from the moment of catch till it reaches the consumer, so as to ensure good quality and long storage life of the shrimp. The maintenance of high personal and environmental hygiene as well as proper heating and cooking will improve fresh shrimp quality and prevent food-borne diseases

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